

CLAIMS

What is claimed is:

1. A process for producing para-hydroxystyrene comprising:
  - a) providing an enzyme source having para-hydroxycinnamic acid decarboxylase activity, said enzyme source comprising a polypeptide having the amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4;
  - b) contacting said enzyme source with para-hydroxycinnamic acid in a biphasic reaction medium comprising an aqueous phase and an extractant, said extractant being a water-immiscible organic solvent selected from the group consisting of toluene, methyl decanoate, 2-undecanone, dichloromethane, hexane, 2-decanol, 4-decanol, 3-decanone, 4-decanone, 1-nonanol, 2-nonanol, 2-heptanol and mixtures thereof, to form para-hydroxystyrene which is extracted into the extractant of the biphasic reaction medium;
  - c) separating the extractant from the aqueous phase; and
  - d) optionally, recovering the para-hydroxystyrene from the extractant.
2. A process according to Claim 1 wherein the enzyme source having para-hydroxycinnamic acid decarboxylase activity is selected from the group consisting of: purified enzyme, cell-free extract, wildtype host cells, recombinant host cells, treated wildtype host cells, and treated recombinant host cells.
3. A process according to Claim 2 wherein the wildtype host cell is selected from the group consisting of *Lactobacillus plantarum* and *Bacillus subtilis*.
4. A process according to Claim 2 wherein the recombinant host cell is selected from the group consisting of bacteria, yeasts, plant cells, and algae.
5. A process according to Claim 4 wherein the recombinant host cell is selected from the group consisting of *Escherichia*, *Salmonella*, *Bacillus*, *Lactobacillus*, *Acinetobacter*, *Streptomyces*, *Methylobacter*, *Rhodococcus*, *Pseudomonas*, *Rhodobacter*, *Synechocystis*, *Aspergillus* and *Arthrobotrys*.
6. A process according to Claim 4 wherein the recombinant host cell is selected from the group consisting of *Saccharomyces*,

*Zygosaccharomyces*, *Kluyveromyces*, *Candida*, *Hansenula*,  
*Debaryomyces*, *Pichia*, *Mucor*, and *Torulopsis*.

7. A process according to Claim 4 wherein the recombinant host cell is selected from the group consisting of soybean, rapeseed, pepper,  
5 sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, grass seed crops, sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees, and forage grasses.

10 8. A process according to Claim 1 wherein the enzyme source is immobilized.

9. A process according to Claim 1 wherein the extractant is present in the biphasic reaction medium in an amount from about 5% to about 70% by volume.

15 10. A process according to Claim 1 wherein the extractant is present in the biphasic reaction medium in an amount from about 20% to about 50% by volume.

20 11. A process according to Claim 1 wherein the extractant is separated from the aqueous phase by use of a gravity settler, a centrifuge, or a hydrocyclone.

12. A process according to Claim 1 wherein the enzyme source is recovered from the aqueous phase of the biphasic reaction medium after the separating of step (c) for reuse.

25 13. A process according to Claim 12 wherein the enzyme source is recovered from the aqueous phase using a method selected from the group consisting of filtration, ultrafiltration, nanofiltration, and centrifugation.

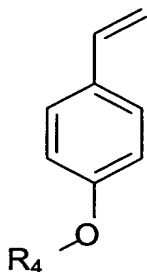
30 14. A process according to Claim 1 wherein the recovering of step (d) is accomplished by means selected from the group consisting of evaporation, distillation, adsorption by resins, and adsorption by molecular sieves.

15. A process according to Claim 1 wherein after step (d), the extractant is optionally added back to the biphasic reaction medium.

35 16. A process according to Claim 1 wherein the aqueous phase after step (c) is optionally added back to the biphasic reaction medium.

17. A process according to Claim 1 wherein the para-hydroxystyrene is chemically derivatized in the extractant to form a derivatized compound.

18. A process according to Claim 17 wherein the derivatized compound is defined by the general formula:



5 wherein R<sub>4</sub> is selected from the group consisting of: methyl, t-butyl, alkyl, silyl ethers, allyl, t-butoxy carbonyl, hydroxyethoxy, acetoxy, formate, glycidyl, benzoate, phenylcarbonate, tetrahydropyran, benzyl, and poly(ethylene oxide).

10 19. A process according to Claim 18 wherein the derivatized compound is para-acetoxystyrene.

20. A process for producing para-hydroxystyrene comprising:

- 15 a) providing a production host which produces para-hydroxycinnamic acid;
- b) growing the production host in a fermentation medium wherein the production host produces para-hydroxycinnamic acid into the fermentation medium;
- 20 c) contacting the fermentation medium from step (b) with an enzyme source having para-hydroxycinnamic acid decarboxylase activity, said enzyme source comprising a polypeptide having the amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, in a biphasic reaction medium comprising the fermentation medium and an extractant, said extractant being a water-immiscible organic solvent selected from the group consisting of toluene, methyl
- 25 decanoate, 2-undecanone, dichloromethane, hexane, 2-decanol, 4-decanol, 3-decanone, 4-decanone, 1-nonanol, 2-nonanol, 2-heptanol and mixtures thereof, to form para-hydroxystyrene, which is extracted into the extractant of the biphasic reaction medium;
- 30 d) separating the extractant from the fermentation medium; and

- e) optionally recovering the para-hydroxystyrene from the extractant.

21. A process according to Claim 20 wherein the production host and insoluble materials are removed from the fermentation medium prior to the contacting of step (c).

22. A process according to Claim 21 wherein the production host and insoluble materials are removed from the fermentation medium by filtration or centrifugation.

23. A process according to Claim 20 wherein the production host is selected from the group consisting of *Escherichia*, *Methylosinus*, *Methylomonas*, *Pseudomonas*, *Streptomyces*, *Corynebacterium*, and *Rhodobacter*.

24. A process according to Claim 20 wherein the enzyme source having para-hydroxycinnamic acid decarboxylase activity is selected from the group consisting of: purified enzyme, cell-free extract, wildtype host cells, recombinant host cells, treated wildtype host cells and treated recombinant host cells.

25. A process according to Claim 24 wherein the wildtype host cell is selected from the group consisting of *Lactobacillus plantarum* and *Bacillus subtilis*.

26. A process according to Claim 20 wherein the recombinant host cell is selected from the group consisting of bacteria, yeasts, plant cells, and algae.

27. A process according to Claim 26 wherein the recombinant host cell is selected from the group consisting of *Escherichia*, *Salmonella*, *Bacillus*, *Lactobacillus*, *Acinetobacter*, *Streptomyces*, *Methylobacter*, *Rhodococcus*, *Pseudomonas*, *Rhodobacter*, *Synechocystis*, *Aspergillus* and *Arthrotrix*.

29. A process according to Claim 26 wherein the recombinant host cell is selected from the group consisting of *Saccharomyces*, *Zygosaccharomyces*, *Kluyveromyces*, *Candida*, *Hansenula*, *Debaryomyces*, *Pichia*, *Mucor*, and *Torulopsis*.

30. A process according to Claim 26 wherein the recombinant host cell is selected from the group consisting of soybean, rapeseed, pepper, sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, grass seed crops,

sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees, and forage grasses.

31. A process according to Claim 20 wherein the enzyme source is immobilized.

5           32. A process according to Claim 20 wherein the extractant is present in the biphasic reaction medium in an amount from about 5% to about 70% by volume.

          33. A process according to Claim 20 wherein the extractant is present in the biphasic reaction medium in an amount from about 20% to  
10       about 50% by volume.

          34. A process according to Claim 20 wherein the extractant is separated from the fermentation medium by use of a gravity settler, a centrifuge, or a hydrocyclone.

          35. A process according to Claim 20 wherein the enzyme source is  
15       recovered from the fermentation medium after the separating of step (d) for reuse.

          36. A process according to Claim 35 wherein the enzyme source is recovered from the fermentation medium using a method selected from the group consisting of filtration, ultrafiltration, nanofiltration, and  
20       centrifugation.

          37. A process according to Claim 20 wherein the recovering of step (e) is accomplished by means selected from the group consisting of evaporation, distillation, adsorption by resins, and adsorption by molecular sieves.

25           38. A process according to Claim 20 wherein after step (e), the extractant is optionally added back to the biphasic reaction medium.

          39. A process according to Claim 20 wherein the fermentation medium after step (c) is optionally added back to the biphasic reaction medium.

30           40. A process according to Claim 20 wherein the para-hydroxystyrene is chemically derivatized in the extractant to form a derivatized compound.

          41. A process according to Claim 40 wherein the derivatized compound is para-acetoxystyrene.

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